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Review

Heat stress effects on crop performance and tools for tolerance breeding

Efectos de estrés por calor sobre la performance de los cultivos y herramientas para el mejoramiento genético de tolerancia

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ABSTRACT

Abiotic stress is one of the most common causes of crop deficit and loss and hence an important area of study. Moreover, concerns regarding global climate change over past decades mean the study of different abiotic stresses appears to be essential if its effects are to be mitigated. The current review covers the effects of heat stress on crop performance, the response crops make when subjected to this stress and the development of tools designed to breed for stress tolerant crops. Distinct levels of the problem are considered, from the morphological/anatomical, through the physiological and to the biochemical/molecular. The study of heat shock proteins (HSPs), quantitative trait loci (QTLs) identification and the relationship between metabolomics (OMICS) and heat stress are given special consideration.

Keywords
heat stress • heat shock proteins (HSPs) • lipid peroxidation • quantitative trait loci (QTLs) • metabolomics (OMICS) • antioxidant enzymes

RESUMEN

Considerando el estrés abiótico como una de las causas más comunes de déficit y las pérdidas de las cosechas resulta muy importante investigar a fondo esta temática. Por otra parte, teniendo en cuenta que los cambios climáticos globales son uno de los problemas que se enfrentan en las últimas décadas, el estudio de los diferentes tipos de estrés abiótico es esencial. La presente revisión se refiere a los efectos del estrés por calor sobre la performance de los cultivos, la respuesta generada por ellos sujetos a este estrés y el desarrollo de herramientas diseñadas para el mejoramiento genético de cultivos tolerantes al mismo. Se consideran distintos niveles de la problemática, desde lo morfológico/anatómico, a través de lo fisiológico y a lo bioquímico/molecular. Se presta especial consideración al estudio de las proteínas de choque térmico (HSPs), a la identificación de los loci de caracteres cuantitativos (QTLs) y a la relación entre la metabolómica (OMICS) y el estrés térmico.

Palabras clave
estrés térmico • proteínas de choque térmico (HSPs) • peroxidación lipídica • loci de caracteres cuantitativos (QTLs) • metabolómica (OMICS) • enzimas antioxidantes

INTRODUCTION

Consideration of abiotic stresses in crop species is of vital importance due to the widespread presence of such stresses on agricultural land, the probable increase in their severity and incidence due to global climactic change and other anthropogenic activities, and the frequent deleterious effects such stresses have on crop productivity. These effects are the result of processes that can be observed at different levels of plant behavior, *i.e.* morphological, physiological and biochemical/molecular changes (145).

At the morphological level, abiotic stress can cause altered shoot, root and leaf growth, as well as developmental changes that result in altered life cycle duration and fewer and/or smaller organs (132, 145). Physiological processes are also affected, such as photosynthetic rate, respiration and the partitioning of assimilates to different organs within the plant (132). At the cellular level, cell membranes can be damaged,

thylakoid structures disorganized, cell size reduced, stomatomal guard cell function altered, degree of cellular hydration modified and programmed cell death promoted. And finally, at the biochemical/molecular level, effects include enzyme inactivation, the production of reactive oxygen species, osmotic damage, changes in primary and secondary metabolite profiles, changed water and ion uptake or movement and altered hormone concentrations.

These effects are in general potentially deleterious for plant performance and plants possess an array of strategies for combating them aimed at maintaining growth and productivity (132, 145), within the limits of ecology, timing, severity and crop stage (53).These strategies include an antioxidative defense system, the activation of protein synthesis for specific tasks involved in ameliorating the effects (such as heat shock proteins, HSPs), ethylene production, detoxification, osmoprotection, stabilization of enzymes and membranes, avoidance of stresses that occur at fixed times during the year and the ability to acclimatize to stresses. The antioxidative defense system protects against reactive oxygen species (18, 100). Ascorbate, glutathione and alpha-tocopherol act as antioxidants in aerobic cells, and carotenoids have important antioxidant effects in photosynthetic systems (52, 77, 100). In addition, an antioxidant enzyme system for scavenging the toxic oxygen species acts in various plant cell compartments, which includes catalase (CAT), superoxide dismutase (SOD). Halliwell-Asada pathway enzymes, ascorbate peroxidase (APx), peroxidise (POD), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) (18, 39, 100). Both natural and artificial stress provoke increased production of toxic oxygen species, and, in response, the capacity of the antioxidative defence system is increased (39, 68, 100).

Breeding for tolerance to stress is based upon harnessing or generating genetic variation in the processes that plants possess for ameliorating the consequences of stress. Although the task has been hampered by the frequent lack of high throughput screening methods to identify tolerant individuals (116), progress is being made through conventional breeding (59) the introduction of novel variation from wild relative species (59) and the use of molecular marker methods to identify quantitative trait loci (QTLs) for tolerance (74), coupled with an increasing understanding of the genetic basis of physiological traits involved in stress tolerance . Additionally, novel variation is being generated by transgenesis (74).

In this review, we cover the effects of heat stress on crop performance and the processes by which plants respond, including the contribution of HSP, QTL and metabolomics studies to its understanding, as well as consideration of the effect of heat stress in the presence of drought stress, given the frequency with which these stresses occur together.

THE EFFECTS OF HEAT STRESS

With industrialization, natural environment deterioration and climate change, heat stress has become an increasingly important factor affecting crop growth, and it is

thought that crop production may be severely affected by an increase in mean global temperature (47, 53). For example, models indicate that a mean temperature rise of 1°C could reduce wheat yields by 10% in some regions (1a) which could be highly relevant in the context of estimated projections for global mean surface temperature rise of up to 4.8°C published by the Intergovernmental Panel on Climate Change (1b), due to multiple factors that appear to include increases in the concentration of greenhouse gases such as carbon dioxide, methane, nitrous oxide and chlorofluorocarbons.

Heat stress-induced decrease of the duration of developmental phases leading to fewer and smaller organs, lower light perception due to a reduced life cycle and altered carbon assimilation is of major importance for cereal yields losses (53, 137). High temperature may slow down or prevent germination, depending on plant species and stress intensity, and, at later stages, may adversely affect photosynthesis, respiration, water relations and membrane stability, as well as modulate levels of hormones and primary and secondary metabolites. Furthermore, throughout plant ontogeny, enhanced expression of a variety of heat shock and other stress-related proteins, and enhanced production of reactive oxygen species (ROS) constitute major plant responses to heat stress (144).

High temperatures reduce photosynthesis by changing the structural organization of thylakoids (66, 144). In general, it is evident that high temperature considerably affects anatomical structures not only at the tissue and cellular levels, but also at the sub-cellular level. The cumulative effects of all these changes under may result in poor plant growth and productivity (144). Plants can adapt to changing environmental conditions by a series of strategies aimed at the maintaining of cellular metabolism, molecular activities, growth and development, through a variety of molecular networks that allow a rapid and efficient sensing of the stress, resulting in the triggering of a response activation (114).

Earlier studies have revealed the nature of heat stress effects on mature, well- developed green leaves (7, 29, 42, 62, 94, 98). However, the impact of heat stress on the development of the photosynthetic system during seedling establishment and leaf growth has not been studied extensively. Exposure to high temperature adversely affects germination and seedling emergence in wheat (6, 29) and retards shoot and leaf growth in *Lolium* (29, 107) and sorghum (29, 60). The impact of heat stress on seedling growth and leaf development has also been established based upon pigmentation sensitivity (28, 68, 92) and Photosystem II (PS II) function in wheat (29, 97, 98). Genotypes of wheat exhibited considerable variation in their sensitivity to heat stress (6, 7, 29). Differences in the sensitivity of chloroplast photoreactions to heat stress, however, could not be established detected between wild and cultivated wheat species (29, 119) or between temperate and tropical cereals cultivars, including wheat (29, 56, 131).

Studies intended to establish cultivar response to temperature suggest that it is not possible to generalize the relationship between temperature and sensitivity of all developmental phases and in all wheat varieties (29, 110, 119) prior to assessment of wheat cultivars for thermotolerance.

Moreover it has been demonstrated that the higher the temperature the plant tolerates, the more protective machineries are involved (53). Heat stress has a complex impact on cell function, suggesting that many processes are involved in thermotolerance. Some processes may be specific to basal thermotolerance, others may be induced during acquired thermotolerance, and many may be involved in both. High temperatures are known to affect membrane-linked processes due to alterations in membrane fluidity and permeability (5, 76, 127).

Some studies have demonstrated that the responsiveness of cultivars to elevated temperature during greening of wheat seedlings is differential, as judged from the analysis of lipid peroxidation, pigmentation, light-induced changes in chlorophyll a fluorescence and photosynthetic electron transport (29).

Heat-induced alterations in enzyme activity can lead to imbalance in metabolic pathways and can cause complete enzyme inactivation due to protein denaturation (62, 71, 138). Membrane and protein damage lead to the production of active oxygen species that cause heat induced oxidative stress (30, 31, 45, 75, 76).

Heat stress causes, like other abiotic stresses, a series of complex morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (132, 145). Among the many deleterious effects described are growth reduction, decreased photosynthetic rate, increased respiration, assimilate partitioning towards the fruits, osmotic and oxidative damage, reduced water and ion uptake/movement, and cellular dehydration. On the other hand, plants activate stress-responsive mechanisms, such as shifts in the aforementioned protein synthesis detoxification, osmoprotection, and stabilization of enzymes and membranes (132).

Moreover, supraoptimal root zone temperatures severely reduce root elongation and increase average root diameter (112, 132). Increased ethylene production also seems to play a role as well in this stress response (1, 132). It is known that heat stress may provoke multiple mineral deficiencies (P and Fe) in roots and shoots, which can both increase ethylene production (132, 139, 147).

Heat can also promote programmed cell death (76, 138, 141). In plants, these different types of damage translate into reduced photosynthesis, impaired translocation of assimilates and reduced carbon gain, leading to altered growth and reproduction (49, 76).

THE RELATION BETWEEN HEAT SHOCK PROTEINS (HSPs) AND THERMOTOLERANCE

The best-characterized of the above mentioned aspects of acquired thermotolerance is the production of heat shock proteins (HSPs) (76, 143). During acclimation, plants, like other organisms, induce massive transcription and translation of HSPs. These proteins are proposed to act as molecular chaperones to protect cellular proteins against irreversible heat-induced denaturation and to facilitate refolding of

heat- damaged proteins (17, 76). Plants cope with heat stress in a complex manner, where HSPs might play a central role in the complex cellular network (10, 53). Moreover, based upon results showing differential expression of carbohydrate biosynthesis and metabolic pathway enzymes, it was also inferred that plants need high amounts of energy to cope with heat stress. As previously mentioned, the disorganization of thylakoid structure can occur upon exposure to high temperature, resulting in reduction of photosystem I (PS I) and PS II activity (53, 142).

The transcription of HSP genes is regulated by heat stress transcription factors that carry out their functions through recognizing and binding with heat stress elements conserved in the promoters of heat stress-inducible genes (10, 53, 101).

At the cellular and molecular levels, the synthesis of HSPs is essential in preventing or minimising the generally deleterious effects of high temperatures. Plant responses to high temperatures are mediated by both their inherent ability to survive, known as basal thermotolerance, and their ability to acquire thermotolerance after acclimation. A major aspect of the acclimation response involves the expression of HSP genes. Moreover, the accumulation of mitochondrial HSP transcripts appears to be related to the acquisition of thermotolerance. (116).

The synthesis of these proteins is known to be part of the stress tolerance strategy resulting in the ability of plants to cope with heat stress (58, 110). As a result of HSP production, many physiological characteristics are improved, such as membrane stability (2, 116), the use of water and nutrients, and assimilate partitioning (116, 134). Thermotolerance is a multigenic trait and the genetic variability in basal and acquired tolerance needs to be assessed by using appropriate approaches. Screening techniques are required to study genetic variability in the stress response and wheat genotypes have been screened based upon cell membrane thermal stability (CMS) by measuring electrolyte diffusion resulting from heat-induced cell membrane leakage (38, 115, 134).

Genetic evidence has established that the HSPs 100 family proteins are essential for the acquisition of thermotolerance in plants. Loss-of-function mutants of Hsp101 in *Arabidopsis* (*Arabidopsis thaliana*; hot1) (57, 58, 76) and maize (*Zea mays*) (76, 99) are unable to acquire thermotolerance at several different growth stages. The phenomenon is a complex process in which different sets of genes are involved in acquired and basal thermotolerance and in thermotolerance at different plant growth stages (76).

Each member of the HSP gene family might have a distinct function in the molecular response to high temperature. However, each HSP subfamily also has a known chaperone activity mediated by a common mechanism of action. All the chaperones contributing to this network, acting in concert, are responsible for the general protective effect of HSPs (116, 144). There is substantial evidence that induction of HSP gene expression plays a role in the acquisition of thermotolerance (116).

Transcriptomic and proteomic studies on rice responses to high temperature have obtained valuable results. Genes (53, 151) and proteins (53, 84) responsive to high temperature during rice grain development were identified. A total of 63 proteins in rice were affected in abundance by high temperatures, 52 of which were identified by MALDI-TOF mass spectrometry and sorted into nine functional groups (53, 78). The two most abundant groups were photosynthesis/ photorespiration- related proteins and heat shock proteins.

For the former, eleven protein spots were identified. Two isoforms of the ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) large subunit (D18 and D19) and glyceraldehyde-3-phosphate dehydrogenase (D15) were down-regulated by high temperature. Three oxygen-evolving complex (OEC) proteins (D22, D26 and D27) were down-regulated at each high temperature tested. RuBisCO activase precursor (U13) was upregulated at each high temperature. Three photorespiration-related proteins, chloroplast glutamine synthetase precursor (U14), glycine dehydrogenase (D1) and glycine cleavage system H protein (U36) were also regulated by high temperature. Fourteen protein spots were identified as HSPs (53).

In a study carried out in rice by Han *et al.*, 21 up-regulated protein spots were identified in rice subjeted to high-temperature, and these proteins were further analyzed to reveal the protective mechanisms of rice seedlings. Most of the proteins were related to photosynthesis and photorespiration, or were anti-stress proteins and HSPs (53).

In this study RuBisCO activase precursor (U13) was up-regulated at each high temperature (53). High temperature can reduce the activation state of RuBisCO (27, 53), which is often attributed to thermolabilility and loss of activity of RuBisCO activase under high- temperature stress (27, 53).

One method to measure thermotolerance is the demonstration of oxidative damage by testing for thiobarbituric acid accumulation (TBARS). High levels of TBARS correlate with the oxidative damage of membrane lipids. The sensitivity of green cells to heat- induced oxidative degradation of lipids (29, 94) is due to the presence of an unusually high percentage of poly-unsaturated fatty acids in thylakoid membrane lipids (21, 29).

Some studies in wheat have been carried out and there is accumulating evidence that HSPs play an important role in thermotolerance in this species. A wheat ditelosomic line lacking the long arm of chromosome 1B, DT1BS, was able to acquire thermotolerance at lower induction temperatures and was therefore better protected from heat stress damage (88, 104). In contrast, a ditelosomic line missing the long arm of chromosome 7D was heat-sensitive and exhibited reduced expression of some HSPs; it was concluded that this arm carries genes necessary for the induction of several HSPs and the consequent capacity to acquire thermotolerance (88, 104).

Application of exogenous compounds or transgenic manipulations has been widely used to determine molecular factors critical for thermotolerance.

In situations where thermal stress exists there is a high probability of finding drought stress. For this reason it is necessary to compare both abiotic stresses. It is well known that the combination of salt, drought and temperature stress in wheat cultivars affects in various ways shoot and root length, and the proportions of different metabolites. Regarding shoot length it as been reported that this is not affected by the combination of thermal and drought stresses (66). Later in this article we include a section on the joint effects of heat and drought stress.

QUANTITATIVE TRAIT LOCI (QTLs)

There is a strong necessity to elucidate the genetic and molecular basis of thermotolerance in crops in order to identify beneficial alleles and genes for use in molecular breeding programmes aimed at producing better cultivars in the future. As we have seen, thermotolerance is not generally controlled by single genes, but by different sets of genes at different stages of the life cycle in various tissues. This requires the analysis of quantitative trait loci (QTLs), which are regions of the genome that control a quantitative trait (QT), *i.e.* one controlled by considerable numbers of genes.

Statistical methods are used to observe a significant linkage between phenotype in a segregating population and genotype at each locus of a linkage map. When transcript or metabolite profiles are used as QTs, these are referred to as expression quantitative trait loci (eQTLs) and metabolite quantitative trait loci (mQTLs), respectively (74).

The most obvious application for genomic technologies has been in the area of gene discovery through the forward genetics or positional cloning approach. However, several other factors limit gene discovery by positional cloning. Perhaps the most important is the reliability of the phenotyping assay that is used to detect the gene or quantitative trait locus (QTL). For traits of low heritability or subject to genotype by environmental interaction, it can be difficult to unequivocally assign a phenotype (74).

Molecular markers can be used for QTL location through segregation and association mapping to identify useful alleles in both cultivated varieties and wild relatives. Although association mapping is intrinsically more powerful than classical genetic linkage mapping because it scrutinizes the results of thousands of generations of recombination and selection, most of the QTL data available up to date are based on segregation mapping.

QTL analysis to dissect high-temperature tolerance in cereals was used by Ottaviano *et al.* (105) who detected six QTLs accounting for 53% of the genetic variability for inherent cellular membrane thermostability (CMS) in a maize recombinant inbred line (RIL) population. Later, in the same RIL population, five QTLs for pollen ability to germinate at high temperature and six QTLs for pollen tube growth at high temperature were identified (38, 39, 84).

A significant challenge in gene discovery based on genetic studies is the final identification of the gene or regulatory sequence responsible for the phenotype. Positional cloning provides very useful genetic data, but it does not conclusively identify the key sequence variant associated with the target phenotype.

Omics technology can help to identify likely candidates that underlie genetic position and elucidate the biological role or process that determines the gene effect. RNA (transcriptomics), protein (proteomics) and metabolite (metabolomics) levels can be assessed for the parental lines. These data can provide direct support for positional cloning by delivering information on genes in the target region associated with an mRNA, protein or metabolite shift linked to the trait of interest.

For example, if the target trait is a QTL associated with stress tolerance, transcriptomics will reveal genes in the region that are up or down-regulated in response to the stress; similarly, protein profiling permits a view of changes in protein abundance or modification in response to the stress. Metabolomics data might indicate that the region is associated with a major change in the cosentration of an osmoprotectant, which suggests that those genes are involved in the biosynthesis (35, 70).

Metabolite, protein and transcript profiles can also be directly mapped onto a segregating population to provide information on loci that control gene expression levels, protein modification or levels of a particular secondary metabolite. The QTLs associated with such traits are known as eQTL (as mentioned above), protein (pQTL) or mQTL (74).

The genes that show differential expression between the recombinants can be genetically mapped to see if their location is linked to the targeted QTLs. For example, 88 genes that are differentially expressed during drought stress have been co-located with QTLs of drought tolerance in maize, and further selected as candidates for positional cloning (74, 91).

THE ROLE OF METABOLOMICS

Plants possess a huge range of chemical repertoires fitting the needs of a highly variable and generally hostile global environment (13, 50, 51). This metabolite richness, reputed to extend to as many as 200,000 compounds in the plant kingdom (34, 51, 103), is also reflected in our plant-based foods that have been reported to contain approximately 25,000 different metabolites of which approximately 7,000 are volatile components (43, 44, 51). It is this richness and our desire for the most holistic understanding of plant metabolism that are the driving forces behind the discipline of metabolomics, which seeks to define the metabolite profile of a particular plant cell, tissue or organ under specific genotypic or environmental circumstances.

However, natural metabolic diversity and a lack of unifying principles to help us detect and identify compounds are likely to be major analytical challenges for many years to come (19, 51).

Metabolomics clearly has much to offer in developing new insights into the regulation of plant metabolism, but these technological limitations mean a full characterization represents a major task, even though for a single species the number of metabolites may be only a few thousand (the estimate for *Arabidopsis* is ca. 5000) (32).

Nonetheless, metabolomics is well placed to detect the pathway driving expression of a trait, potentially enabling breeders, by selecting on the basis of biochemical markers, to combine pathways to traits of quality in high-yielding backgrounds with good tolerance to biotic and abiotic stress. Metabolomic profiling can give an instantaneous snapshot of the overall physiology of a cell, and its integration with transcriptomics and proteomics should lead to a more complete picture of a living organism's biology (71) and more precise selection tools for the breeder.

Plant physiological responses to environmental stress involve changes in transcriptional regulation and post-translational protein modification, which can lead to alterations in the metabolite profile and the consequent production of a specific physiological response phenotype. Frequently in nature, various stress factors are present simultaneously, such as drought, high temperature and osmolality, meaning it is not always clear which factor or factors are behind the response (1c). Careful experimental work aimed at distinguishing the effect of these factors on plant metabolism is therefore required.

The comparison of transgenic and control plants offers an excellent opportunity of interpreting microarray and other OMICS data because of the generally single genetic difference between the genotypes. A similar situation is encountered with knock-out mutants, especially those that have been backcrossed to the parental line to remove irrelevant genetic lesions. Furthermore targeted studies of maize kernels have demonstrated the impact of factors such as developmental stage (32, 133), environment and farming practice (31, 52, 53), and genetic background and growing seasons (32, 120, 122) on the natural variability of metabolites.

OMICS analysis has already been used to study natural periodicities such as the cell cycle (63, 126) and day-night cycles (126, 128). Special clustering algorithms exist to deal with the ability of mutations and stresses to alter the time-dependence of metabolism, growth and development (126, 149). Such alterations in time dependence are familiar in plant physiology and agronomy from using degree-day units to account for the impact of temperature on growth (15, 126) and the stress-time index to schedule irrigation (9, 126). Omics platforms expand these analyses to link physiological and molecular knowledge to the more conventional phenotypic and genetic analyses (35).

Molecular plant breeding is built around predictions of phenotype based on genotypes. The reliability of these predictions is derived from measurements of phenotypic performance in large segregating populations, followed by the application

of statistical procedures based on quantitative genetic theory. Analysis of complex traits has been supported by developments in statistical and modeling techniques for phenotypic data, which have been generated from field and controlled environment studies (35, 90).

Application of this index has permitted identification of the stages of crop development in which the interaction between the genotype and the environment is strongest. This information can then be used to identify the components of the crop response that offer the greatest response to breeding and selection for stress (23, 74).

Regarding heat in particular, this stress, which tends to be detected due to alterations in the plasma membrane, alters protein and nucleic acid stability, cytoskeleton structure and enzymatic efficiency, leading to acute metabolic imbalance. It also leads to metabolically induced impairment of electron transport chains and ROS production such as NADPH oxidase bound to the membrane. Furthermore the photosynthetic system is another target of this stress (PSII, the oxygen-evolving complex, the ATP system and carbon assimilation (1c). As implied earlier, metabolomic analysis of these changes should lead to new understanding and new selection tools for future breeding programs.

JOINT PRESENCE OF DROUGHT AND HEAT STRESS

Anatomical changes under high ambient temperatures and drought respectively are generally similar at the whole plant level, there is a general tendency of reduced cell size, closure of stomata and curtailed water loss, increased stomatal and trichomatous densities, and greater xylem vessels of both root and shoot (11, 147).

Where heat and drought stress occur simultaneously, as they often do, (3, 91, 117), they generally result in more extreme detrimental effects than would each stress separately, as shown by results from both field crops and model plants (126, 129, 146). Nonetheless, it has been observed that their joint presence can alter plant metabolism in novel ways compared to each applied individually (124, 126).

For example, it has been suggested that heat stress can mitigate the toxicity of proline to cells, since plants subjected solely to drought accumulate proline, whereas plants subjected to a combination of drought and heat stress replace proline with sucrose as the major osmoprotectant (124, 126).

Since there were many similarities between the responses of *Arabidopsis* (124, 126) and tobacco (*Nicotiana tabacum*) (123, 126) to this stress combination, this mode of defense response might be conserved among different plant species. Another example of an interesting interaction is the observation that waterlogging stress does not cause important damage at low temperatures, but it may be highly harmful at high temperatures.

In addition to the well known genes specifically induced by heat and drought stresses (*i.e.* HSPs and dehydrins) a number of novel durum wheat sequences have been revealed the up-regulation of these genes when heat stress is combined with drought confirms the involvement of this gene family in abiotic stress response and two new members of this gene family were identified that respond to the combination of both heat and drought stress (117).

Heat and drought stress can, of course, occur in the presence of other stresses. In a study carried out on wheat seedlings, the researchers demonstrated the effect of combination of different abiotic stresses with different stresses having been applied in different experiments, for example, salt, drought and heat stress (66).

Indeed, drought, salinity, high temperatures and oxidative stresses are often interconnected and produce cellular damage. For example, drought and / or salinization initially manifest osmotic stress, leaving to the disruption of homeostasis and ion distribution in the cell. Oxidative stress that is often combined with high temperature, salinity or drought stress may cause denaturation of functional and structural proteins.

Kuznetsov & Shevyakova (73) suggested that the development of thermotolerance in salt tolerant cells could be caused by the more active heat shock protein synthesis induced by the abrupt increase in temperature (70, 73). Some studies for example Keleş & Öncel (70) indicate that the negative effects of salt stress on shoot and root elongation were forced by low and high temperature stress. On the other hand, while CAT activity in cytoplasm was depressed by a combination of high temperature and salt stress, GR activity in chloroplasts was increased (70). In this study, the activity of CAT decreased by 80% in a high temperature-drought combination in wheat leaves.

The heavy stress conditions that cause loss of CAT activity induce the activities of the Halliwell-Asada pathway enzymes such as GR and APx (46, 70, 93).

Further research is required to determine the responses of plants under environmental stress combinations. For example, in durum wheat, the molecular response to individual heat, drought or other abiotic stresses has been studied (8, 112, 114, 116, 117), but a similar understanding in the simultaneous presence of heat and drought stress is critical for the development of new breeding strategies aimed at the production of durum wheat cultivars characterized by enhanced tolerance to multiple environmental stresses (3, 117, 140).

DISCUSSION

Since abiotic stresses have been repeatedly reported as acutely affecting the productivity of crops in general and wheat in particular, more detailed study is required to elucidate the fundamental processes involved in these effects, in order to provide further tools for protecting crops in the future. For example, in wheat, different stages in the cycle should be covered, such as tillering, heading and maturity, and including

the seedling stage in which relatively little work has been carried out, analogous to studies of heat stress carried out in rice seedlings (53).

In the case of our current research in wheat (108), further research is required in order to fully understand the system of antioxidant enzymes in wheat, since this system appears to be an important component of a seedling's response to adverse climates, including heat and drought stress.

As previously mentioned, wheat cultivars vary in their physiological behavior when subjected to high temperatures under different conditions. We believe that when exposing plants to heat stress in controlled environment cabinets, humidity control must be very strict. Without such regulation, we have observed mortality of all the seedlings included in trials carried out in hydroponics in which we subjected the plants to 35°C.

Furthermore, tolerance to a combination of different stress conditions, particularly those that mimic the field environment, should be one of the principal focuses of future research programs aimed at developing crops with enhanced tolerance to stress in the field, including transgenic crops (121).

CONCLUSION

Understanding the effects of global climate change appears to be crucial in deciding which geographical regions and temporal periods should be used for future crop cultivation. The simultaneous analysis of different abiotic stresses will be essential in achieving this and providing the means to mitigate any adverse effects of this change, since natural and human-made climate change generate multiple abiotic stresses.

Plants that are affected by various abiotic stresses react differently as a result such as the reduction in organ size or a change in the antioxidant enzyme system, and while drought and heat are often studied separately, in nature they often occur together, meaning it is essential to carry out joint studies.

Furthermore, and related to the above when we consider high temperature stress, we must always consider the heat shock proteins HSPs, since these are responsible for cell membrane stability, the use of water and nutrients by the plant and other physiological functions. The effects of heat stress can also be analyzed by the TBARS technique that measures lipid peroxidation in polyunsaturated acids of the plasma membrane. Considering that many of the physiological effects of the plant are reflected by a change in the plasma membrane, this technique should prove of particular value.

Through the application of these approaches in wheat it should be possible to achieve an understanding of the effects of abiotic stresses on crop performance and mitigate those effects accordingly.

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